CALCIUM EFFECTS ON FREE AND CHROMATIN-BOUND RNA POLYMERASE II REACTIONS

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1. Introduction

In 1960, Weiss described the RNA polymerase activity in rat liver chromatin as an 'aggregate enzyme' [1]. Ca^{2+} has since been considered to be an inhibitory cofactor for this enzyme. However, details of the Ca^{2+} effect on RNA polymerase activity are not yet completely understood.

Here, effects of Ca^{2+} on the RNA polymerase II reactions were studied in relation to the enzyme form (free or chromatin-bound enzyme). Results indicate that initiation step of free RNA polymerase II is inhibited by Ca^{2+}, whereas the elongation step of template-engaged enzyme is stimulated. In addition, these differential effects of Ca^{2+} were not observed for the α-amanitin resistant enzyme. The apparent discrepancy between these results and those in [1] was investigated.

2. Materials and methods

2.1. *Nuclear isolation and preparation of free and chromatin-bound RNA polymerase*

Isolation of nuclei was performed by a modification of the method in [2] from Sprague-Dawley rat liver. Sucrose (2.1 M) containing 3 mM $MgCl_2$ was used instead of calcium-containing medium for the isolation. Free and chromatin-bound RNA polymerase I and II from nuclei were partially purified with DEAE–Sephadex A-25 column chromatography according to [3]. These enzyme preparations were free of RNA degradation activity as judged by enzyme kinetic studies.

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2.2. *RNA polymerase assay*

RNA polymerase was assayed in several different ways:

(1) The standard assay was performed as in [4]. Enzyme solution (100 μl) was added to a reaction mixture giving 250 μl final vol. containing 50 mM Tris–HCl (pH 7.9), 0.5 mM dithiothreitol, 50 mM $(NH_4)_2SO_4$, 0.5 μCi [^{3}H]UTP (1 Ci/mmol, New England Nuclear) and 10 μg native calf thymus DNA. After incubation at 30°C for 30 min, the reaction was stopped by the addition of cold 10% trichloroacetic acid, and acid-insoluble materials were collected on a Whatmann GF/F filter. The filters were washed with cold 5% trichloroacetic acid and 95% ethanol, dried and counted in a scintillation counter.

(2) The initiation assay was performed in two steps:
(i) With a high concentration of non-labeled NTPs (2.5×10^{-4} M) and with various added calcium levels in 200 μl total vol. Other conditions were the same as in our standard assay [4]. After incubation at 30°C for 5 min, the mixture was chilled in ice.
(ii) An elongation reaction was performed in the presence of labeled UTP (1 μCi) in 250 μl final vol. $CaCl_2$ was adjusted to 10 mM and heparin (2 mg/ml) was added to inhibit re-initiation [5]. Incubation was at 30°C for 25 min after which incorporated radioactivity was measured as in our standard assay.

(3) The elongation assay also consisted of two steps:
(i) Same conditions as the above initiation assay in 200 μl with incubation at 30°C for 5 min.

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(ii) The reaction was then chilled in ice and various levels of $CaCl_2$, labeled UTP (1 μCi) and 0.5 mg heparin were added to give a final volume of 250 μl. Further incubation was carried out at 30°C for 25 min.

Endogenous template-dependent enzyme activity bound to chromatin was assayed without exogenous calf thymus DNA using our standard assay condition. Chromatin extracted from nuclei with 0.34 M sucrose as in [3] contained no free enzyme activity.

Each enzyme activity reported is the average value of duplicate assays.

In addition, the α-amanitin resistant enzyme activity was estimated in the presence of α-amanitin (1 μg/ml) under the same conditions as above-mentioned RNA polymerase II assay methods.

3. Results and discussion

Although Weiss reported the drastic inhibition of RNA polymerase activity at 1–2 mM $CaCl_2$ [1] the solubilized free and chromatin-bound RNA polymerase II assayed here showed only mild inhibition, ~50% of control activity, at 5 mM $CaCl_2$ (fig.1 (□),2 (□)). In order to determine which step of the RNA polymerase II reaction was inhibited by Ca^{2+}, the initiation and elongation were subsequently assayed. The initiation step of free RNA polymerase II was inhibited by ~35% at 2.5 mM (fig.1 (○)). On the contrary, the elongation step was stimulated by ~50% of control activity at the same [Ca^{2+}] (fig.1 (△)).

Then, to investigate the effect of Ca^{2+} on chromatin-bound RNA polymerase II, the solubilized bound enzyme was also assayed for initiation and elongation activity. As observed with the free enzyme, the solubilized bound enzyme was inhibited by Ca^{2+} in the standard (fig.2 (□)) and initiation (fig.2 (○)) assays, but stimulated in the elongation assay (fig.2 (△)) at 1 mM $CaCl_2$.

It therefore appears that Ca^{2+} exhibits an inhibitory effect on the initiation step of free RNA polymerase II and a stimulatory effect on the elongation reaction of the template-engaged enzyme. In contrast

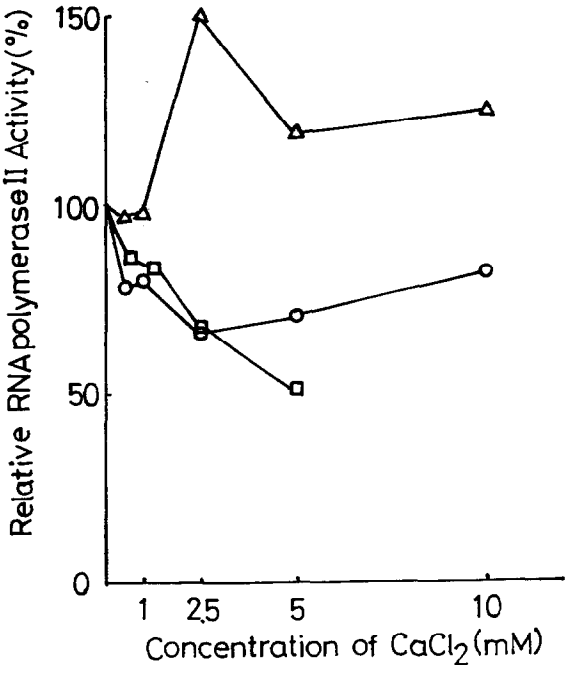

Fig.1. Effect of Ca^{2+} on the free RNA polymerase II reaction. Standard (□——□), initiation (○——○) and elongation (△——△) assay of a partially purified free RNA polymerase II were done as in section 2. Control activity of standard, initiation and elongation assays was 5922, 967 and 584 cpm, respectively.

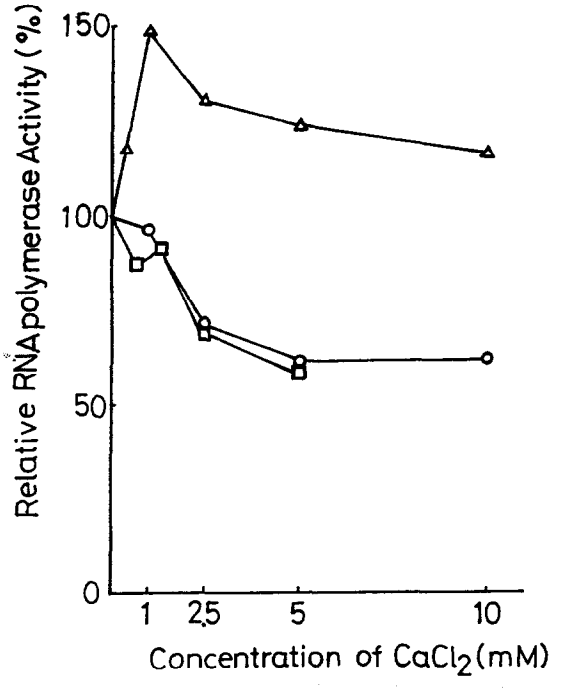

Fig.2. Effect of Ca^{2+} on the solubilized chromatin-bound RNA polymerase II reaction. Standard (□——□), initiation (○——○) and elongation (△——△) assay of a partially purified chromatin-bound RNA polymerase II were done as in section 2. Control activity of standard, initiation and elongation assays was 1839, 1161 and 583 cpm, respectively.

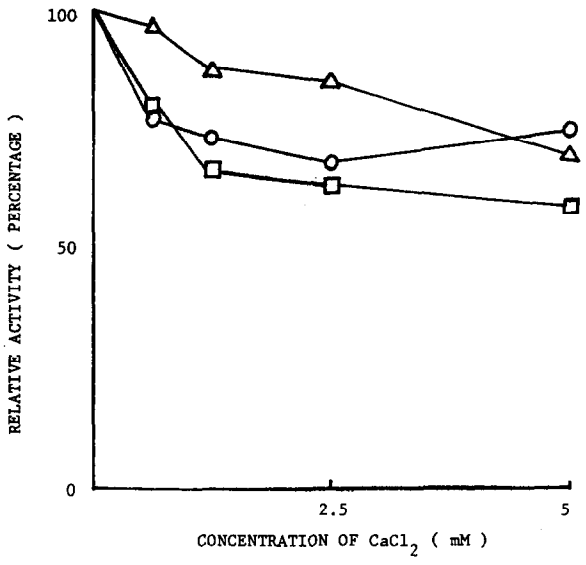

Fig.3. Effect of Ca^{2+} on the RNA polymerase I reaction. Enzyme was prepared by DEAE–Sephadex A-25 chromatography as in section 2 from chromatin. Control activity of standard (□——□), initiation (○——○) and elongation (△——△) assays was 1250, 520 and 430 cpm, respectively.

to these results obtained for RNA polymerase II, the α-amanitin-resistant enzyme showed only inhibitory effects by Ca^{2+} under the above conditions (fig.3). The differential Ca^{2+} effect seems therefore to be specific for RNA polymerase II.

The discrepancy between these results and the strong inhibitory effect of Ca^{2+} in [1] was studied specifically. When the various assay conditions were compared and analyzed, Ca^{2+} showed its strong inhibitory effect on the α-amanitin-resistant enzyme specifically in the absence of Mn^{2+}. This phenomenon was found for both the standard assay of solubilized RNA polymerase I and the α-amanitin-resistant activity of the endogenous chromatin-bound enzyme (fig.4a,b). The latter is almost identical to the 'aggregate enzyme' [1] and his assay mixture did not contain Mn^{2+}. These results suggest that the strong inhibitory effect of Ca^{2+} found in [1] was due to inhibition of the α-amanitin-resistant enzyme in the absence of Mn^{2+}. This effect was not observed for the RNA polymerase II reaction.

Acknowledgement

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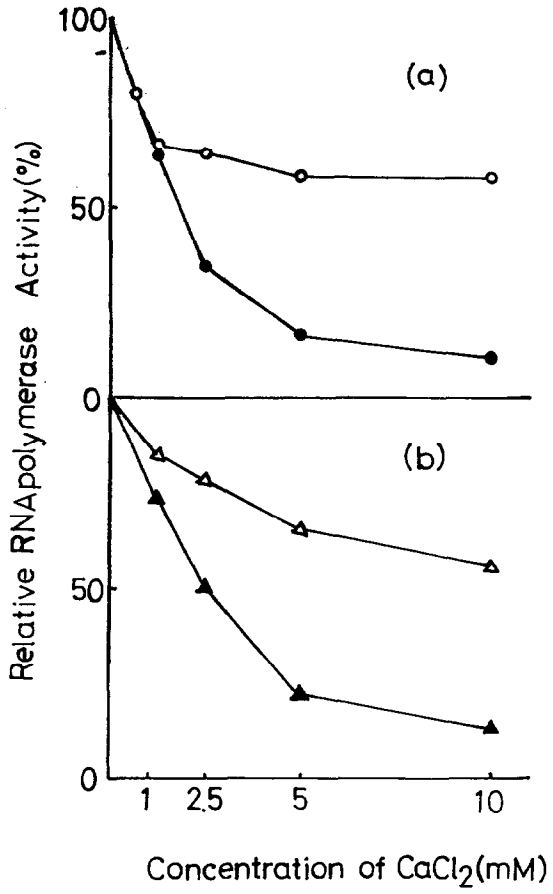

Fig.4. Effect of Ca^{2+} on the α-amanitin-resistant RNA polymerase reaction in the absence of Mn^{2+}. (a) The assay for RNA polymerase I was performed under the same conditions as the standard assay in fig.3. Control (○——○) and manganese-deficient (●——●) assays were performed. The control activity of these assays was 2643 and 1299 cpm, respectively. (b) Endogenous template-dependent α-amanitin-resistant activity of chromatin was assayed under control (△——△) and manganese-deficient conditions (▲——▲) in the presence of α-amanitin (1 μg/ml) as in section 2. The control activity of the assays was 3468 and 1299 cpm, respectively.

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